REMARKS

Claims 23-28 are pending in this Application. Claim 28 has been withdrawn and claims 23-27 stand rejected under 35 U.S.C. § 103(a) as being obvious over Nilsson[1] in view of Sukosol[2] (claims 24 and 25) or as being obvious over Nilsson and Sukosol in view of Salzman[3] *et al.* (WO 01/040280) ("Salzman") and Fruitstone[4] (claims 23, 24, 26 and 27).

REJECTION OF CLAIMS 24 AND 25

Claim 24 is directed to an agglutination reagent for rapid and early detection of typhoid. As amended, claim 24 recites that the reagent consists essentially of a plurality of carboxylated latex particles consisting essentially of an antibody specific to a Flagellin gene of *Salmonella typhi*. The particles are stored in a storage buffer. Claim 25 depends from claim 24 and further defines the particles.

Nilsson is directed to a system for detecting protein using two different monoclonal antibodies for human chorionic gonadotropin ("hCG").[5] There are two different monoclonal antibodies covalently bound to the latex particles.[6] Once the latex particles are created, Nilsson teaches washing the particles with Tris-BSA and blocking the carboxyl group with Tris-HCL.[7] When a test reagent is mixed with an hCG containing sample, an immune

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[1] Nilsson *et al.* "Microparticles for selective protein determination in capillary electrophoresis," ELECTROPHORESIS, (2001) 22: 2384-2390 ("Nilsson").

[2] Sukosol *et al.*, "Fusion protein of *Salmonella typhi* flagellin as antigen for diagnosis of typhoid fever," ASIAN PACIFIC J. OF ALLERGY AND IMMUN., (1994) 12:21-25 ("Sukosol").

[3] WO 01/040280 to Salzman *et al.* ("Salzman").

[4] U.S. Patent No. 4,379,847 to Fruitstone *et al.* ("Fruitstone").

[5] Nilsson at abstract.

[6] Nilsson at abstract.

[7] Nilsson at page 2385.

complex is formed.[8] The complex is separated from the latex particles using capillary electrophoresis and detected by a UV-Vis detection system.[9] Since Nilsson discusses using an instrument to detect the hCG, the system is confined to the laboratory, and cannot be used in the field. A capillary electrophoresis is different from an agglutination system.

Nilsson also fails to disclose the recited antibody, an agglutination reagent, and the storage buffer.

Sukosol used a 900 base pair gene sequence specific to the *Salmonella typhi* flagellin gene to make a fusion protein with a GST tag in a vector for detection of the antibody (IgM) in serum samples of individuals suspected to have typhoid or related infection.[10] Sukosol does not disclose using the *Salmonella typhi* flagellin gene product for making an antibody, binding the antibody to a latex particle or using the antibody in an agglutination reagent.

Point I. The cited references do not teach or suggest a plurality of latex particles coated with a single antibody.

There is no reason why one would reasonably expect Nilsson's invention to work if the second antibody is removed. Assuming that one was motivated to combine the references, one would create a monoclonal antibody raised against Sukosol's 900 base pair gene sequence specific to *Salmonella typhi*. This antibody would be reacted with Nilsson's latex particle. The resulting reagent would contain two types of particles, each with a different antibody.

In contrast, claims 24 and 25 recite that the reagent consists essentially of a plurality of carboxylated latex particles coated with a single antibody. Since there is no reason to remove the second antibody from Nilsson, the invention is not obvious over the cited references.

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[8] Nilsson at abstract.

[9] Nilsson at abstract.

[10] Sukosol at page 23, column 3.

Point II. There is no teaching or reason to use the recited storage buffer.

Neither Nilsson nor Sukosol teaches the storage buffer recited in claim 24. This deficiency is not overcome by Fruitstone because there is no reason to expect that Fruitstone can be applied to Nilsson's method.

Fruitstone discloses a suspending medium for immunohematologic reactions like antibody screening, antibody identification, crossmatches, antiglobulin testing, blood grouping, etc.[11] While, in passing, it mentions that "the solution may also be used in other types of immunologic reactions, such as latex particle agglutination tests,"[12] it does not provide sufficient evidence to enable one of ordinary skill to reasonably believe that this would be expected to work. It fails to provide any experiment details with respect to detection of viruses or bacteria. This information is necessary to provide one a reasonable expectation that the medium would not induce false positives, or, more specifically, the likelihood of false positives in latex particle agglutination tests.

REJECTION OF CLAIMS 23, 24, 26 AND 27

Claim 23 is directed to a process for the preparation of an agglutination reagent for rapid and early detection of typhoid comprising preparing antibody specific to a Flagellin gene of *Salmonella typhi*, preparing a latex particle suspension, and coating of a plurality of latex particles with the antibody. The antibody is prepared by raising a hyper immune sera against a purified protein encoded by a Flagellin gene specific to *Salmonella typhi*.

The latex particle suspension is prepared in part by mixing 1% carboxylated latex particles and 40 mM 2-N morpholinoethane sulphonic acid (MES) buffer of pH 5.5 to 6.0 in a ratio of 1:1, washing twice with 20 mM MES buffer of pH 5.5 thereby forming a plurality of

[11] Fruitstone at col. 4, lines 26-35.

[12] Fruitstone at col. 4, lines 31-35.

washed latex particles. 1-ethyl-3 (3-dimethyl-amino propyl) carbodiimide hydrochloride (EDC) in 20 mM MES buffer of pH 5.5 is added to the washed latex particles.

The washed latex particles are coated with the antibody fraction. The reaction is stopped with 1M glycine (pH 11.0), and then the antibody coated latex particles are washed with a buffer comprising 50 mM glycine, pH 8.5; 0.03% surfactant and 0.05 % sodium azida.

As discussed above, claim 24 is directed to an agglutination reagent for rapid and early detection of typhoid. As amended, claim 24 recites that the reagent consists essentially of a plurality of carboxylated latex particles coated with an antibody specific to a Flagellin gene of *Salmonella typhi*. The particles are stored in a storage buffer. Claims 26 and 27 depend from claim 24.

Nilsson is directed to a system for detecting protein using two different monoclonal antibodies for human chorionic gonadotropin ("hCG").[13] There are two different monoclonal antibodies covalently bound to the latex particles.[14] Once the latex particles are created, Nilsson teaches washing the particles with Tris-BSA and blocking the carboxyl group with Tris-HCL.[15] When a test reagent is mixed with hCG containing sample, an immune complex is formed.[16] The complex is separated from the latex particles using capillary electrophoresis and detected by UV-Vis detection system.[17] Since Nilsson discusses using an instrument to detect the hCG, the system is confined to the laboratory, and cannot be used in the field. A capillary electrophoresis is different from an agglutination system.

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[13] Nilsson at abstract.

[14] Nilsson at abstract.

[15] Nilsson at page 2385.

[16] Nilsson at abstract.

[17] Nilsson at abstract.

Nilsson does not disclose several of the limitations recited in claim 23. Namely, Nilsson does not disclose: an antibody specific to *Salmonella typhi* Flagellin gene product because Nilsson is directed to hCG, washing the polyclonal monospecific coated latex particle with MES because Nilsson teaches washing with Tris-BSA, and blocking the carboxyl group with Tris-HCl, or an agglutination reagent because Nilsson teaches capillary electrophoresis. Nilsson also fails to disclose several limitations recited in claims 24, 26, and 27. Namely, it fails to disclose: the recited antibody, an agglutination reagent, and the storage buffer.

Sukosol used a 900 base pair gene sequence specific to the *Salmonella typhi* flagellin gene to make a fusion protein with a GST tag in a vector for detection of the antibody (IgM) in serum samples of individuals suspected to have typhoid or related infection.[18] Sukosol does not disclose using the *Salmonella typhi* flagellin gene product for making an antibody, binding the antibody to a latex particle or using the antibody in an agglutination reagent.

Salzman generally is directed to a polypeptide derived from flagellin polypeptides used to generate an immune response to gram-negative bacteria.[19] Salzman used a portion of a *Salmonella muenchen* specific flagellin gene product to prepare an antibody.[20] The gene product is comprised of less than 160 amino acids, which also match the flagellin amino acid sequence of other gram negative bacteria. Salzman used GST as a tag with the gene sequence to make the fusion protein.[21] Antibodies raised against this protein will not only react with clinical samples of *Salmonella muenchen* and other gram-negative bacteria, but will also react with parasitic infections caused by *Schistosoma japonicum*. Therefore, Salzman does not teach a polyclonal antibody, nor a monospecific antibody because the antibody generated, according to Salzman's disclosure, will not be specific to *Salmonella muechen*. Salzman also does not disclose (1) the

[18] Sukosol at page 23, column 3.

[19] Salzman at abstract.

[20] Salzman at pages 11-21.

[21] Salzman at page 31.

recited antibody raised against Salzman's protein will react with *Salmonella muenchen,* other gram-negative bacteria and parasites such as *Schistosoma japonicum*, (2) using a latex particle, or (3) the recited reagents.

Fruitstone is directed to a suspending medium for use in immunological reactions, specifically immunohematologic reactions.[22] Although Fruitstone mentions in passing that its solution can be used in other immunologic reactions such as latex particle agglutination tests,[23] it does not provide any experiments or other details for the use of its suspending medium in such reactions.

Point I. There is no reason to eliminate Nilsson's second antibody

There is no reason why one would reasonably expect Nilsson's invention to work if the second antibody is not present. Assuming that one was motivated to combine the references, one would create a monoclonal antibody raised against Sukosol's 900 base pair gene sequence specific to *Salmonella typhi*. This antibody would be reacted with Nilsson's latex particle. The resulting product would contain at least a first and a second latex particle, each coated with a different antibody.

In contrast, claim 23 recites "... coating a plurality of latex ~~particle~~ particles with said antibody specific to said Flagellin gene of *Salmonella* typhi ...." Likewise, claims 24, 26 and 27 recite that the reagent consists essentially of latex particles that are coated with an antibody, not two different antibodies. Since there is no reason for one to have removed the second antibody from Nilsson, the invention is not obvious over the cited references.

[22] Fruitstone at col. 1, lines 10-14.

[23] Fruitstone at col. 4, lines 31-33.

Point II. There is no reason to substitute Nilsson's blocking and washing steps with the recited ones.

Additionally, the cited references do not teach the recited blocking and washing steps, and there is no reason to substitute Nilsson's washing and blocking steps with the recited washing step. Nilsson teaches blocking the residual activated carboxyl groups with 0.1 M Trus-HCl, pH 8.0 containing 0.2% BSA, and washing the antibody coated latex particles with Tris-BSA. However, claim 23 recites that the reaction between the latex particle coated and the polyclonal-monospecific antibody is stopped with 1M glycine (pH 11.0), and the polyclonal-monospecific antibody coated latex particle is washed with 50 mM glycine, pH 8.5; 0.03% surfactant and 0.05% sodium azide.

Nilsson teaches using BSA to activate the particle and to stop the reaction. In contrast, claim 23 recites that the reaction is stopped with 1M glycine (pH 11.0), and that the coated latex particles are washed with a washing buffer comprised of 50 mM glycine, pH 8.5; 0.03% surfactant and 0.05% sodium azide. The concentrations of reagents, pH and temperature play a critical role in *in vitro* immunological tests (see J. EXP. MED. (1924) vol. 39(2): 265; also see J. Exp. Med. (1926) vol. 44(5): 667; see also J. INFECT. DIS. (1933) vol. 53(1); see also J. IMMUNOL. (1948) vol. 58: 229; see also J. IMMUNOL. (1973) col. 111: 478; see also ESSENTIALS OF IMMUNOL. AND SEROL. (2002): 204-205).

Point III. There is no teaching or reason to use the recited storage buffer.

Neither Nilsson nor Sukosol teaches the storage buffer recited in claim 24 and further defined in claim 26. This deficiency is not overcome by Fruitstone because there is no reason to expect that Fruitstone can be applied to Nilsson's method.

Fruitstone discloses a suspending medium for immunohematologic reactions like antibody screening, antibody identification, crossmatches, antiglobulin testing, blood grouping,

etc.[24] While, in passing, it mentions that "the solution may also be used in other types of immunologic reactions, such as latex particle agglutination tests,"[25] it does not provide sufficient evidence to enable one of ordinary skill to reasonably believe that this would be expected to work. It fails to provide any experiment details with respect to detection of viruses or bacteria. This information is necessary to provide one a reasonable expectation that the medium would not induce false positives, or, more specifically, the likelihood of false positives in latex particle agglutination tests.

Point IV. The secondary evidence rebuts any *prima facie* case of obviousness.

Notwithstanding the reasons set-forth above, even assuming that a *prima facie* case of obviousness has been established, the secondary evidence of long-felt but unresolved need, failure of others and commercial success rebuts the rejection. Typhoid is one of the most prevalent diseases afflicting countries in tropical regions of the world. It is also a very difficult disease to diagnose. The gold standard for diagnosing Typhoid is by taking a culture. However, this diagnostic test is very time consuming. Other methods include the Widal test, Widal slide agglutination Test, Radioimmunoassay, ELISA based antigen detection methods and commercially available Typhidot™. These methods all have several shortcomings, as listed in Table 1.

Table 1: Shortcomings of Typhoid Tests

Test	Shortcoming
Widal Test	Takes 18-25 hours after 6-7 days of enteric fever[26]
Widal Slide Agglutination Test	Take 1-3 min. after 6-7 days of enteric fever[27]

[24] Fruitstone at col. 4, lines 26-35.

[25] Fruitstone at col. 4, lines 31-35.

[26] Specification at page 2, lines 5-6.

Test	Shortcoming
Culture Test	Takes 3-14 days after first day of fever[28]
ELISA based test	Takes 8 hours after 6-7 days of enteric fever
Radioimmunoassay	Very complicated and involves the use of radioactive material, which is not available in many countries where Typhiod is a health concern.[29]

In contrast to these shortcomings, the claimed invention provides a process for the preparation of an agglutination reagent that can be used to detect typhoid early and rapidly with 100% specificity. The recited invention takes approximately 1-2 minutes to develop a color positive sample.[30] Since the invention relies on seeing a color, an untrained person can read the results. Thus, the invention is fast and easy to use in comparison to the prior art.

Furthermore, the invention can be used to detect typhoid at the onset of infection. The prior art is not capable of doing this.

Additionally, the invention is highly specific and sensitive for detecting typhoid. In contrast, the Widal test cross-reacts with other febrile organisms and other organisms within the Enterobacteriaceae family.[31] Also, it provides false positives when administered to a person who has been vaccinated, or when the appropriate baseline level of typhoid for a region is not known (typhoid, being an endemic, causes background level of antibody in endemic areas).[32]

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[27] Specification at page 2, lines 5-6.

[28] Specification at page 2, line 20 to page 3, line 16

[29] Specification at page 3, line 17 to page 4, line 3.

[30] Specification at page 7, lines 17-19.

[31] Specification at page 1, lines 24-26.

[32] Specification at page 2, lines 1-4.

The culturing technique is also a low sensitivity test for typhoid (40 – 60 %) because there are very few organisms in circulation (as low as 1 / ml), which leads to false negatives.[33]

The agglutination reagent is stable for more than nine months at 4°C.

Finally, a leading Indian company has already taken interest in this invention.

Thus, there is a long-felt, but unresolved need for a fast and easy typhoid diagnostic test that can detect typhoid in its early stages. The prior art only teaches tests that are either time consuming (e.g. culture tests), or can only detect typhoid 6-7 days after enteric fever has been observed. Others who have tried to address this problem have failed by either developing a test that is time consuming, or can only detect typhoid 6-7 days after enteric fever.

In view of this secondary evidence, Applicants have rebutted any *prima facie* case of obviousness set-forth by the Examiner. In the absence of evidence to the contrary, the Applicants respectfully request that this rejection be reconsidered and withdrawn.

Point V. There is no reason to use an agglutination test.

Claim 23 is directed to agglutination tests, whereas Nilsson is directed to a capillary electrophoresis detection system. In Nilsson's system, when the antibody-coated latex particle reacts with the protein, the complex is separated from single latex particles using capillary eletrophoresis and detected by ultra violet diode array detection or laser-induced fluorescence imaging detection.[34]

Sukosol does not overcome this deficiency because it is not directed to an agglutination system. Sukosol is solely directed to the existence of a 52kDa antigen of

[33] Specification at page 3, lines 8-10.

[34] Nilsson at page 2384, col. 1.

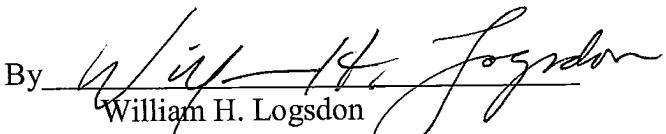
Salmonella typhi detected by monoclonal antibodies via Western blot and ELISA assays. These methods do not permit one to detect *Salmonella typhi* with the naked eye.

CONCLUSION

Due to the differences discussed above, a combination of the cited references do not result in the recited invention. For these reasons, Applicants respectfully request reconsideration and withdrawal of the objections and rejections, allowance of pending claims 23-27, and rejoinder of claim 28.

Respectfully submitted,

THE WEBB LAW FIRM

By 

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